

WHAT IS CLAIMED IS:

1. A bioconjugate comprising:

a tether comprising a segment capable of recognizing and interacting with β -secretase;

5 a fluorescer comprising a plurality of fluorescent species, the fluorescer conjugated to a first location on the tether; and

a quencher conjugated to a second location on the tether;

wherein the segment capable of recognizing and interacting with the target biomolecule is located between the first and second locations on the tether, and

10 wherein the plurality of fluorescent species are associated with one another such that the quencher is capable of amplified super-quenching of the fluorescer.

2. The bioconjugate of Claim 1, wherein the segment capable of recognizing and interacting with β -secretase comprises the peptide sequence:

SEVNLDAEF (SEQ ID NO:1).

15 3. The bioconjugate of Claim 1, wherein the fluorescer comprises a polymer or oligomer comprising a plurality of fluorescent repeating units.

4. The bioconjugate of Claim 1, wherein the fluorescer comprises a solid support associated with a plurality of fluorescent species.

20 5. The bioconjugate of Claim 4, wherein one or more quenchers are each linked to the solid support through a reactive tether.

6. The bioconjugate of Claim 4, wherein the solid support is selected from the group consisting of: streptavidin coated spheres; polymer microspheres; silica microspheres; organic nanoparticles; inorganic nanoparticles; magnetic beads;

magnetic particles; semiconductor nanoparticles; quantum dots; membranes; slides; plates; and test tubes.

7. The bioconjugate of Claim 1, wherein the fluorescer is selected from the group consisting of: conjugated polyelectrolytes; fluorescent proteins; biotinylated
5 conjugated polyelectrolytes; functionalized conjugated oligomers; charged conjugated polymers; uncharged conjugated polymers; conjugated polymer blends; and J-aggregated polymer assembly comprising assembled monomers or oligomers.

8. The bioconjugate of Claim 1, wherein the fluorescer is a virtual polymer.

9. The bioconjugate of Claim 1, wherein the fluorescer is a poly(L-lysine)
10 polymer or oligomer having cyanine pendant groups.

10. The bioconjugate of Claim 1, wherein the fluorescer is constructed from an oligosaccharide.

11. The bioconjugate of Claim 4, wherein the fluorescer comprises a fluorescent polymer or oligomer.

12. The bioconjugate of Claim 11, wherein the fluorescent polymer or
15 oligomer is associated with the solid support by: covalent attachment to the solid support; adsorption onto the surface of the solid support; or by interactions between a biotin moiety on the fluorescent polymer or oligomer and an avidin, neutravidin or streptavidin moiety on the solid support surface.

13. The bioconjugate of Claim 1, wherein the fluorescer is conjugated to the
20 tether via a protein molecule.

14. The bioconjugate of Claim 13, wherein the protein molecule is selected from the group consisting of: avidin; neutravidin; and streptavidin.

15. The bioconjugate of Claim 1, wherein the quencher is non-fluorescent.

16. The bioconjugate of Claim 1, wherein the quencher is fluorescent and is capable of reemitting energy absorbed from the fluorescer.

17. A method of assaying for β -secretase activity in a sample comprising:

5 incubating the sample with a bioconjugate comprising a quencher and a ligand conjugated to a tether at first and second locations respectively, wherein the tether comprises a segment between the first and second locations capable of recognizing and interacting with β -secretase;

adding a fluorescer to the incubated sample to form a sample mixture, the fluorescer comprising a plurality of fluorescent species, wherein the fluorescer
10 comprises a moiety capable of binding the ligand of the bioconjugate such that the bioconjugate can bind to the fluorescer, and wherein binding of the fluorescer to the ligand results in amplified superquenching of the fluorescer;

allowing the ligand on the bioconjugate to bind to the fluorescer; and

subsequently measuring the fluorescence of the sample mixture;

15 wherein the amount of fluorescence of the sample mixture indicates the presence and/or amount of β -secretase activity in the sample.

18. The method of Claim 17, wherein the plurality of associated fluorescent species are associated with a solid support.

19. The method of Claim 17, further comprising:

20 adding the fluorescer to a second sample that contains the bioconjugate but has not been incubated with the enzyme to form a control;

measuring the fluorescence of the control; and

comparing the fluorescence of the control to the fluorescence of the sample mixture;

wherein a difference in the fluorescence between the control and the sample mixture is an indication of the presence and/or the amount of β -secretase in the sample.

20. The method of Claim 17, wherein the sample comprises β -secretase and a test compound, the method further comprising:

incubating a second sample containing no test compound with the bioconjugate;

adding the fluorescer to the incubated second sample to form a control;

measuring the fluorescence of the control; and

comparing the fluorescence of the control to the fluorescence of the sample mixture;

wherein a difference in the fluorescence between the control and the sample mixture is an indication of the ability of the test compound to inhibit β -secretase activity in the sample.

21. The method of Claim 17, wherein the ligand is a biotin moiety and the moiety on the fluorescer is avidin, neutravidin or streptavidin moiety.

22. A method of assaying for β -secretase activity in a sample, the method comprising:

incubating the sample with a bioconjugate as set forth in Claim 1; and

measuring the fluorescence of the incubated sample;

wherein the measured fluorescence of the incubated sample is an indication of the presence and/or the amount of β -secretase activity in the sample.

23. The method of Claim 22, further comprising:

measuring the fluorescence of a control; and

comparing the fluorescence of the control to the fluorescence of the incubated sample;

wherein a difference in the fluorescence between the control and the incubated sample is an indication of the presence or amount of β -secretase activity in the sample.

24. The method of Claim 22, wherein the sample comprises β -secretase and a test compound and wherein the ability of the test compound to inhibit β -secretase activity is being assayed.

25. The method of Claim 22, wherein the fluorescer comprises a solid support and wherein the plurality of fluorescent species are associated with the solid support.

26. The method of Claim 25, wherein one or more quenchers are each linked to the solid support through a reactive tether.

27. The method of Claim 25, wherein the solid support is selected from the group consisting of: streptavidin coated spheres; polymer microspheres; silica microspheres; organic nanoparticles; inorganic nanoparticles; magnetic beads; magnetic particles; semiconductor nanoparticles; quantum dots; membranes; slides; plates; and test tubes.

28. A kit comprising:

a fluorescer comprising a plurality of fluorescent species; and

a bioconjugate comprising a quencher and a ligand conjugated to a tether at first and second locations respectively, wherein the tether comprises a segment between the first and second locations capable of recognizing and interacting with β -secretase;

wherein the fluorescer comprises a moiety capable of binding the ligand on the bioconjugate and wherein the plurality of fluorescent species are associated with one another such that the quencher is capable of amplified superquenching of the fluorescer when the ligand is bound to the fluorescer.

5 29. The kit of Claim 28, wherein the plurality of associated fluorescent species are associated with a solid support.

30. The kit of Claim 28, wherein ligand is a biotin moiety.

31. The kit of Claim 28, wherein the moiety capable of binding the ligand is selected from the group consisting of avidin, neutravidin and streptavidin.

10 32. The kit of Claim 28, wherein the segment capable of recognizing and interacting with β -secretase comprises the peptide sequence: SEVNLD AEF (SEQ ID NO:1).

33. A bioconjugate comprising:

15 a tether comprising a segment capable of recognizing and interacting with β -secretase;

 a quencher conjugated to a first location on the tether, the quencher capable of quenching the fluorescence of a fluorescer comprising a plurality of associated fluorescent species; and

 a biotin molecule conjugated to a second location on the quencher;

20 wherein the segment capable of recognizing and interacting with the target biomolecule is located between the first and second locations on the tether and wherein the plurality of fluorescent species are associated with one another such that the quencher is capable of amplified quenching of the fluorescer.

34. The bioconjugate of Claim 33, wherein the segment capable of recognizing and interacting with β -secretase comprises a polypeptide having the sequence:

SEVNLDAEF (SEQ ID NO:1).

35. The bioconjugate of Claim 34, wherein the bioconjugate has a structure represented by:

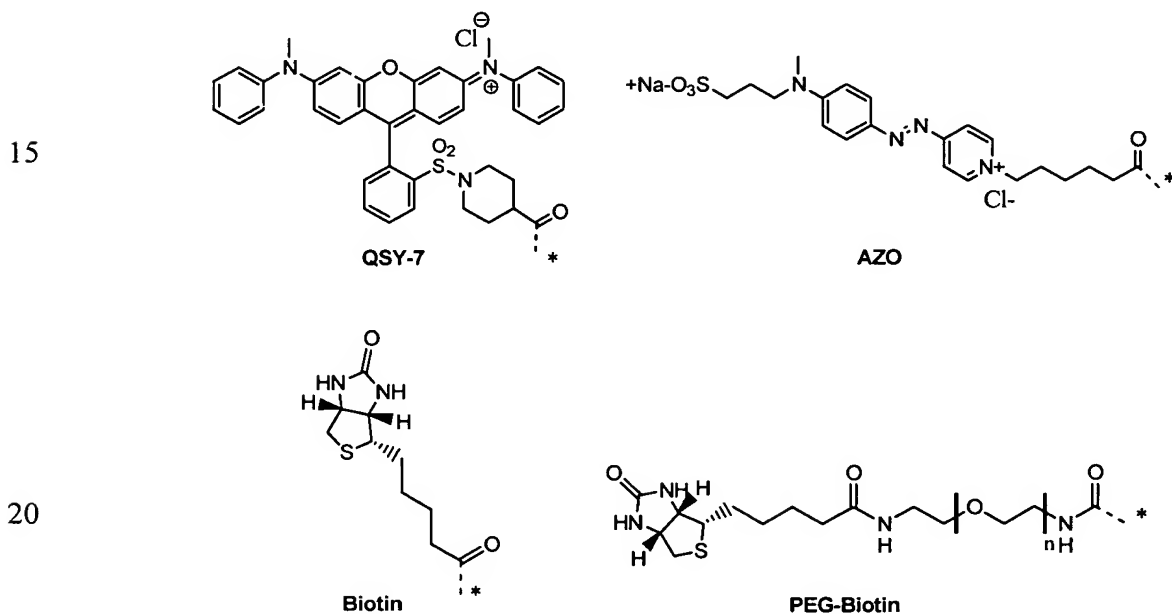
(QSY-7)-TEEISEVNLDAEFK-(N ϵ -Biotin) (SEQ ID NO:2);

(QSY-7)-TEEISEVNLDAEFK-(N ϵ -PEG-Biotin) (SEQ ID NO:3);

(AZO)-TEEISEVNLDAEFK-(N ϵ -Biotin) (SEQ ID NO:4); or

(AZO)-TKKISEVNLDAEFRK-(N ϵ -Biotin) (SEQ ID NO:5);

wherein QSY-7, AZO, Biotin and PEG-Biotin represent moieties having the following structures:



wherein “*” denotes the point of attachment of each moiety to the polypeptide, “N ϵ ” denotes linkage of the biotin moiety or PEG-Biotin moiety to the lysine residue of the

polypeptide through the ϵ -amino group of the C-terminal lysine residue, and wherein the QSY-7 and AZO moieties are attached to the polypeptide through the amino group of the N-terminal threonine residue.

36. A method for assaying for target enzyme activity in a sample comprising:
5 incubating the sample with a bioconjugate comprising a quencher conjugated to a tether, wherein the tether comprises a segment capable of being cleaved by the target enzyme;

adding a fluorescer to the incubated sample to form a sample mixture, the fluorescer comprising a plurality of fluorescent species associated with one another
10 such that a association of the fluorescer with the quencher results in amplified superquenching of the fluorescer; and

allowing the target enzyme to cleave the tether, wherein cleavage of the tether results in a quencher containing bioconjugate fragment that has a greater tendency to associate with the fluorescer than the bioconjugate; and subsequently measuring
15 fluorescence of the sample mixture;

wherein the amount of fluorescence of the sample mixture indicates the presence and/or amount of the target enzyme activity in the sample.

37. The method of Claim 36, wherein the target enzyme is a caspase enzyme.

38. The method of Claim 36, wherein the target enzyme is caspase-3.

20 39. The method of Claim 38, wherein the segment capable of being cleaved by the target enzyme is the peptide sequence:

DEVD (SEQ ID NO:9).

40. The method of Claim 36, wherein association between the quencher containing bioconjugate fragment and the fluorescer comprises coulombic attraction,

41. The method of Claim 36, wherein the fluorescer and the bioconjugate each have an overall negative charge and the quencher containing bioconjugate fragment has a net positive charge.

43. The method of Claim 41, wherein the quencher is a cationic electron or energy transfer quencher.

D-E-V-D-QSY7' (SEQ ID NO:7)

CNCCNC(=O)C1CCN(C1)S(=O)(=O)c2ccccc2-c3cc4c(c(c5ccccc5N(C)C)oc6ccccc6N(C)C)ccc34.[Cl-]

45. The method of Claim 36, wherein the quencher is conjugated to the tether
divalent linker.

46. The method of Claim 45, wherein the divalent linker is a diamine.

47. The method of Claim 36, wherein the fluorescer is a virtual polymer comprising an aggregate of donor cyanine dyes and the quencher is an acceptor cyanine dye and wherein the acceptor, when conjugated to the tether, is unable to form an aggregate with the donor cyanine dyes.

48. The method of Claim 47, wherein the inability of the bioconjugate to form an aggregate with the donor is the result of charge effects or steric effects.

49. The method of Claim 47, wherein the acceptor cyanine dye is fluorescent.

50. The method of Claim 47, wherein the acceptor cyanine dye is fluorescent and wherein the fluorescence of the acceptor is measured.

51. The method of Claim 47, wherein the fluorescence of the donor cyanine dye is measured.

52. The method of Claim 47, wherein the assay is an intracellular assay or an extracellular assay.

53. A method for assaying for target enzyme activity in a sample comprising: incubating the sample with a bioconjugate comprising a fluorescent dye conjugated to a tether, wherein the tether comprises a segment capable of being cleaved by the target enzyme to produce a fluorescent dye containing fragment, and wherein the fluorescent dye containing fragment is capable of forming a dye aggregate which has a different absorption spectra than the bioconjugate and; allowing the target enzyme to cleave the bioconjugate; and measuring the fluorescence of the sample mixture by exciting the sample at a wavelength wherein the dye aggregate absorbs to a greater degree than the bioconjugate;

wherein the amount of fluorescence of the sample mixture indicates the presence and/or amount of target enzyme activity in the sample.

54. The method of Claim 53, wherein the fluorescent dye is a cyanine dye.

55. The method of Claim 54, wherein the fluorescent dye containing fragment is capable of forming a J-aggregate.

56. The method of Claim 53, wherein the target enzyme is a caspase enzyme.

57. The method of Claim 53, wherein the target enzyme is caspase-3.

58. The method of Claim 53, wherein the segment capable of being cleaved by the target enzyme is the peptide sequence:

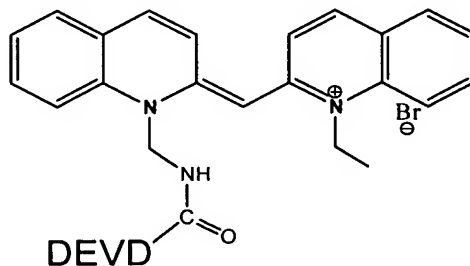
DEVD (SEQ ID NO:9).

59. The method of Claim 58, wherein the bioconjugate has a structure represented by the following formula:

D-E-V-D-Cyanine (SEQ ID NO:8)

wherein "cyanine" represents a cyanine dye moiety and wherein the cyanine dye moiety is conjugated to the α -carboxylic acid group of the C-terminal aspartic acid residue.

60. The method of Claim 59, wherein the bioconjugate has a structure represented by the following formula:



61. A kit comprising:

a fluorescer comprising a plurality of fluorescent species; and
a bioconjugate comprising a quencher conjugated to a tether, wherein the
tether comprises a segment capable of being cleaved by a caspase enzyme;
wherein cleavage of the tether results in a quencher containing bioconjugate
5 fragment that has a greater tendency to associate with the fluorescer than the
bioconjugate and wherein association of the fluorescer with the quencher results in
amplified superquenching of the fluorescer.

62. The kit of Claim 61, wherein the plurality of associated fluorescent
species are associated with a solid support.

10 63. The kit of Claim 61, wherein the caspase enzyme is caspase-3.

64. The kit of Claim 63, wherein the segment capable of being cleaved by a
caspase enzyme is the peptide sequence:

DEVD (SEQ ID NO:9).

65. The kit of Claim 61, wherein the quencher containing bioconjugate
15 fragment associates with the fluorescer via coulombic attraction, hydrogen bonding
forces, van der waals forces, or covalent bond formation.

66. The kit of Claim 61, wherein the fluorescer and the bioconjugate each
have an overall negative charge and the quencher containing bioconjugate fragment
has a net positive charge.

20 67. The kit of Claim 66, wherein the fluorescer is an anionic conjugate
polymer.

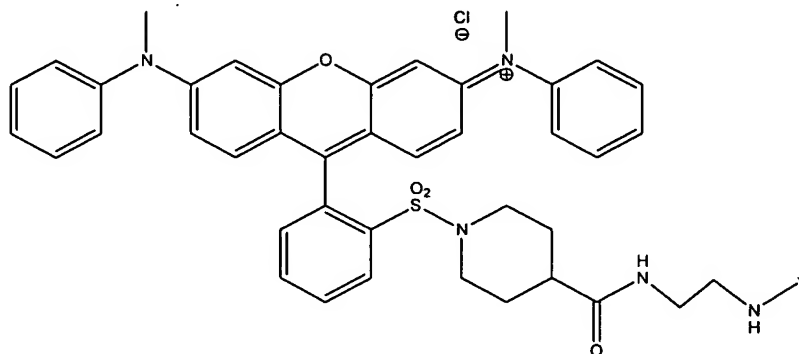
68. The kit of Claim 66, wherein the quencher is a cationic electron or energy
transfer quencher.

69. The kit of Claim 61, wherein the bioconjugate is represented by the

following formula:

D-E-V-D-QSY7' (SEQ ID NO:7)

wherein "QSY7" represents a quencher moiety represented by the following structure:



10 wherein "*" represents the point of attachment of the quencher moiety to the tether and wherein the quencher moiety is conjugated to the tether through the α -carboxylic acid of the c-terminal aspartic acid residue of the tether.

70. The kit of Claim 61, wherein the quencher is conjugated to the tether via a divalent linker.

15 71. The kit of Claim 70, wherein the divalent linker is a diamine.

72. The kit of Claim 61, wherein the fluorester is a virtual polymer comprising an aggregate of donor cyanine dyes and the quencher is an acceptor cyanine dye and wherein the acceptor, when conjugated to the tether, is unable to form an aggregate with the donor cyanine dyes.

20 73. The kit of Claim 72, wherein the inability of the bioconjugate to form an aggregate with the donor is the result of charge effects or steric effects.

74. The method of Claim 72, wherein the acceptor cyanine dye is a non-fluorescent molecule or a fluorescent molecule capable of reemitting energy absorbed from the fluorester.

75. A bioconjugate comprising:

a tether comprising a segment capable of being cleaved by a caspase enzyme;

and

a quencher conjugated to the tether.

5 76. The bioconjugate of Claim 75, wherein the caspase enzyme is caspase-3.

77. The bioconjugate of Claim 76, wherein the segment capable of being cleaved by a caspase enzyme is the peptide sequence:

DEVD (SEQ ID NO:9).

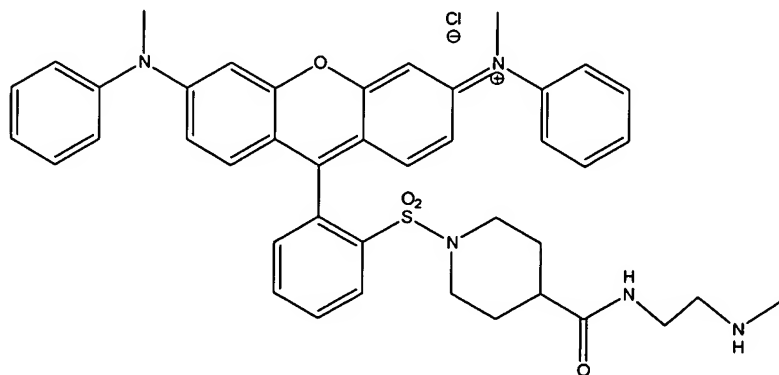
78. The bioconjugate of Claim 75, wherein the quencher is a cationic electron or energy transfer quencher.

79. The bioconjugate of Claim 75, wherein the bioconjugate is represented by the following formula:

D-E-V-D-QSY7' (SEQ ID NO:7)

wherein "QSY7'" represents a quencher moiety represented by the following structure:

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wherein "*" represents the point of attachment of the quencher moiety to the tether and wherein the quencher moiety is conjugated to the tether through the α -carboxylic acid of the c-terminal aspartic acid residue of the tether.

80. The bioconjugate of Claim 75, wherein the quencher moiety is conjugated to the tether through a divalent linker.

81. The bioconjugate of Claim 80, wherein the divalent linker is a diamine.

82. The bioconjugate of Claim 75, wherein the quencher is an acceptor
5 cyanine dye.

83. The bioconjugate of Claim 82, wherein the acceptor cyanine dye is fluorescent.

84. A bioconjugate comprising:

a fluorescent dye conjugated to a tether, wherein the tether comprises a
10 segment which can be cleaved by the target enzyme to produce a fluorescent dye containing fragment, and wherein the fluorescent dye containing fragment is capable of forming a dye aggregate which has a different absorption spectra than the bioconjugate.

85. The bioconjugate of Claim 84, wherein the fluorescent dye is a cyanine
15 dye.

86. The bioconjugate of Claim 84, wherein the fluorescent dye containing fragment is capable of forming a J-aggregate.

87. The bioconjugate of Claim 84, wherein the target enzyme is a caspase enzyme.

88. The bioconjugate of Claim 84, wherein the target enzyme is caspase-3.
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89. The bioconjugate of Claim 88, wherein the segment capable of being cleaved by the target enzyme is the peptide sequence:

DEV D (SEQ ID NO:9).

90. The bioconjugate of Claim 89, wherein the bioconjugate has a structure

represented by the following formula:

D-E-V-D-Cyanine (SEQ ID NO:8)

wherein “cyanine” represents a cyanine dye moiety and wherein the cyanine dye moiety is conjugated to the α -carboxylic acid group of the C-terminal aspartic acid residue of the tether.

91. The bioconjugate of Claim 90, wherein the bioconjugate has a structure represented by the following formula:

